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BASIC AND TRANSLATIONAL—BILIARY

Four Susceptibility Loci for Gallstone Disease Identified in a Meta-analysis of Genome-Wide Association Studies

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BACKGROUND & AIMS: A genome-wide association study (GWAS) of 280 cases identified the hepatic cholesterol transporter *ABCG8* as a locus associated with risk for gallstone disease, but findings have not been reported from any other GWAS of this phenotype. We performed a large-scale, meta-analysis of GWASs of individuals of European ancestry with available prior genotype data, to identify additional genetic risk factors for gallstone disease. **METHODS:** We obtained per-allele odds ratio (OR) and standard error estimates using age- and sex-adjusted logistic regression models within each of the 10 discovery studies (8720 cases and 55,152 controls). We performed an inverse variance weighted, fixed-effects meta-analysis of study-specific estimates to identify single-nucleotide polymorphisms that were associated independently with gallstone disease. Associations were replicated in 6489 cases and 62,797 controls. **RESULTS:** We observed independent associations for 2 single-nucleotide polymorphisms at the *ABCG8* locus: rs11887534 (OR, 1.69; 95% confidence interval [CI], 1.54–1.86; $P = 2.44 \times 10^{-60}$) and rs4245791 (OR, 1.27; $P = 1.90 \times 10^{-34}$). We also identified and/or replicated associations for

rs9843304 in *TM4SF4* (OR, 1.12; 95% CI, 1.08–1.16; $P = 6.09 \times 10^{-11}$), rs2547231 in *SULT2A1* (encodes a sulfoconjugation enzyme that acts on hydroxysteroids and cholesterol-derived sterol bile acids) (OR, 1.17; 95% CI, 1.12–1.21; $P = 2.24 \times 10^{-10}$), rs1260326 in glucokinase regulatory protein (OR, 1.12; 95% CI, 1.07–1.17; $P = 2.55 \times 10^{-10}$), and rs6471717 near *CYP7A1* (encodes an enzyme that catalyzes conversion of cholesterol to primary bile acids) (OR, 1.11; 95% CI, 1.08–1.15; $P = 8.84 \times 10^{-9}$). Among individuals of African American and Hispanic American ancestry, rs11887534 and rs4245791 were associated positively with gallstone disease risk, whereas the association for the rs1260326 variant was inverse. **CONCLUSIONS:** In this large-scale GWAS of gallstone disease, we identified 4 loci in genes that have putative functions in cholesterol metabolism and transport, and sulfonylation of bile acids or hydroxysteroids.

Keywords: Genetics; Risk Factors; SNP; GWAS.

Accounting for a substantial clinical burden in the United States, gallstone disease afflicts 6.3 million men and 14.2 million women between the ages of 20 and 74 years, leading annually to 700,000 cholecystectomies and an economic burden of 6.5 billion dollars.[1] It was hypothesized as early as the 1960s that the composition of bile may play an important role in gallstone formation.[2] Bile is formed by the transportation of cholesterol, bile acids, and other organic molecules such as bilirubin from within the hepatocytes to the biliary canaliculi, and serves as a medium for excretion of lipid-soluble products of metabolism. Precipitation of biliary constituents from their soluble state into their insoluble form initiates the process of gallstone formation. Clinical conditions with chronic hemolytic states such as sickle cell disease frequently have been associated with pigmented gallstones[3] as a result of the increased delivery of unconjugated bilirubin into the bile via hepatocytes.[4] However, the most common (80%–90%) constituent of gallstones retrieved during cholecystectomy surgery or autopsy is biliary cholesterol. Studies that compared the constituents of lithogenic bile and normal bile observed that higher concentrations of cholesterol, or the alterations in relative proportions of other bile components such as bile salts and phospholipids, can result in supersaturation of cholesterol.[2,5] Redinger and Small[6] further showed a correlation between the percentage saturation of biliary cholesterol in various ethnic groups and estimated gallstone prevalence rates in the same population in an ecologic study. Consequently, several lifestyle determinants such as female sex, greater parity, postmenopausal hormone therapy, Native American ancestry, high body mass index (BMI), and dyslipidemia are among the most important risk factors for gallstone disease, primarily because of their influence on cholesterol concentration in the bile.[5,7]

Based on familial clustering of gallstone disease, a 2- to 3-fold increased risk among first-degree relatives,[8–10] and heritability estimates of 25%–29% from twin studies,[10,11] it has been suggested that genetic factors may play an important contributory role in cholelithiasis. More evidence to support this hypothesis was established using experimental crosses of inbred mice strains with varying prevalence of gallstones.[12,13] Quantitative trait loci-based approaches were used to generate a murine gallstone genetic map of several candidate lithogenic (*lith*) loci,[12,14] with the idea that orthologous human *LITH* genes may be predicted owing to homology between human and mouse genomes. These murine *lith* loci co-localized with approximately 7 "likely," and approximately 20 "plausible" candidate genes for gallstone disease, many of which are involved in cholesterol (eg, *ABCG5/ABCG8*) and bile acid (eg, *ABCB11*) synthesis, transport, or metabolism.[13]

The identification of genetic risk factors of gallstone disease in human beings was undertaken in 2007 in a discovery-based genome-wide association study (GWAS) of 280 cases and 360 controls.[15] This study identified and replicated an approximately 2-fold increased risk for carriers of the H-allele of D19H in the hepatic cholesterol transporter gene *ABCG8* (rs11887534; risk allele frequency, ~7%).[15,16] Other studies that examined genetic associations with gallstone disease were based on biological insights of candidate loci or pathways. Buch et al[17] investigated the association of known bilirubin loci[18] with the incidence of gallstone disease, and observed a recessive mode of inheritance at the *UGT1A1* SNP locus rs6742078, finding that carriers of the T/T genotype were predisposed to an increased risk of gallstone disease among men, but not among women.[17] Moreover, a recent study in women, examining associations of approximately 2000 gene-centric loci in known lipid metabolism and obesity pathways,[19] reported additional associations for the glucokinase regulatory protein (*GCKR*) SNP rs1260326 and the *TTC39B* SNP rs686030 with gallstone disease; however, these associations were not replicated.

Although there is strong evidence for genetic contribution toward the risk of gallstone disease, there are few replicated susceptibility loci identified from genome-wide, discovery-based approaches because of the limited size and scope of prior studies. In this study, we therefore conducted a large-scale GWAS meta-analysis in individuals with pre-existing genetic data on more than 2 million genetic variants, to discover additional loci associated with the risk

*Authors share co-first authorship; § Authors share co-senior authorship.

Abbreviations used in this paper: **ARIC, Atherosclerosis Risk in Communities Study; BioVU, Vanderbilt DNA Biobank; BMI, body mass index; CI, confidence intervals; eSNP, expression single-nucleotide polymorphism; eQTL, expression quantitative trait loci; FHS, Framingham Heart Study; GCKR, glucokinase regulatory protein; GCTA, genome-wide complex trait analysis; GRS, genetic risk score; GWAS, genome-wide association studies; HPFS, Health Professionals Follow-up Study; ICD, International Classification of Diseases; lith, lithogenic; MAF, minor allele frequency; NHS, Nurses' Health Study; OR, odds ratio; RPKM, reads per kilobase per million; SHIP, Study of Health in Pomerania; SNP, single-nucleotide polymorphism; WGHS, Women's Genome Health Study; WHI, Women's Health Initiative.**

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